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CONTENT

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SELENITE METABOLISM IN THE MYCELIUM OF THE FUNGUS PHYCOMYCES BLAKESLEEANUS

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ABSTRACT

In this study, mycelium of fungus *Phycomyces blakesleeanus* was exposed to soluble toxic form of selenium, selenite (Se^{+4}), with the aim of determining the flow and products of its biotransformation. Selenite is reduced to Se^0 in the form selenium nanoparticles (SeNPs) and Se^{-2} in the form of methylated volatile selenides. Low concentrations of Se^{+4} in the mycelium form RSSeSR type compounds, which could be a step in the process of SeNPs formation, or incorporation of Se into metabolites such as Se-amino acids.

INTRODUCTION

For many organisms, selenium (Se), which can exist in several oxidation states (-2, 0, +4 and +6) is an essential microelement. Soluble anionic forms of selenium, selanate (Se^{+6}) and selenite (Se^{+4}) can be reductively transformed by microorganisms such as bacteria, fungi and microalgae to insoluble Se^0, often in the form of selenium nanoparticles (SeNPs), and/or selenide (Se^{-2}). In the form of selenide (Se^{-2}), it can be a part of important biomolecules such as selenocysteine and selenomethionine [1], or a part of volatile methylated Se compounds such as dimethylselenide (DMSe) or dimethyldiselenide (DMDSe) [2].

Relatively large number of phylogenetically diverse fungi have the ability to produce SeNPs indicating that the process is probably quite common in fungi, but existing data is mostly technical in nature concentrating almost exclusively on characterization of produced SeNPs and leaving the mechanism of synthesis mostly unknown. One of the mechanisms of selenite toxicity is considered to be production of reactive oxygen species (ROS) that then triggers response of cellular antioxidative systems [3], among which important part is played by thiols such as glutathione. Glutathione is also believed to play a more direct role in reduction of Se^{+4} to Se^0 as proposed by the Painter reaction [4].

P. blakesleeanus is a non-pathogenic filamentous fungus easy to cultivate, characterized by short life cycle and rich yield of mycelium in a short time. Because of these features it was chosen in this study for investigation of Se biotransformation.

METHODS

Mycelium of the fungus *P. blakesleeanus* (Burgeff) (NRRL 1555(-)) was grown to mid-exponential phase (28 h), filtered, washed, and incubated in fresh medium with increasing Na_2SeO_3 (Se^{+4}) concentrations from 0.5 mM, to10 mM for 24 h.

Detection of thiols *in vivo* was performed by room temperature EPR (Bruker EMX Nano X-band) spin trapping with disulphide biradical. Estimation of free thiols was done by determination of mono- and biradical peak ratio in the spectrum of the given sample (M/B).

For ICP-OES measurements, samples were prepared in microwave digestor (ETHOS 1, Milestone, Italy) with 65% HNO_3 and 30% H_2O_2. Selenium quantification was done on Thermo Scientific iCAP 6500 Duo ICP (Cambridge, United Kingdom).

For XANES experiments, aliquots of 5 µL mycelium suspension were placed on 2,5 µm Mylar thin film fixed and attached to the sample holder and freeze-dried overnight. XANES measurements were performed at IAEA X-ray spectrometry experimental station installed at Elettra Sincrotrone Trieste (Trieste, Italy) synchrotron facility.

RESULTS AND DISCUSSION

The mycelium was exposed to Se^{+4} for 24 h at mid-exponential phase. The mycelium itself is yellow-orange due to presence of β-carotene [5], and the toxicity of Se^{+4} is visible already at 0.5 mM treatment, while red coloration appears with 2 mM Se^{+4} and becomes more intense in 5 and 10 mM treatment, indicating the ability of *P. blakesleeanus* for Se^{+4} reduction and formation of amorphous elemental selenium nanoparticles (**Fig 1A**). A pungent odor from treated samples indicated the formation of volatile selenium compounds. Toxicity of Se^{+4} is reflected in biomass reduction at 0.5 mM treatment (**Fig 1B**). Decrease of available thiols (**Fig 1C**) is detected as the decrease in ratio of mono- and biradical peak in the spectrum of the given sample. This ratio was 43.05 for control and didn't change with 0,1 mM treatment (42.23) but halved to 23.55 with 0.5 mM Se^{+4}. It further decreased 10 × to minimal values of 4.31, 4.21, 4.1 and 3.5 with 1-, 2-, 5- and 10-mM Se^{+4}, respectively. This supports hypothesis that thiols participate in Se^{+4} reduction, but the possibility of their decrease due to ROS formation cannot be ruled out.

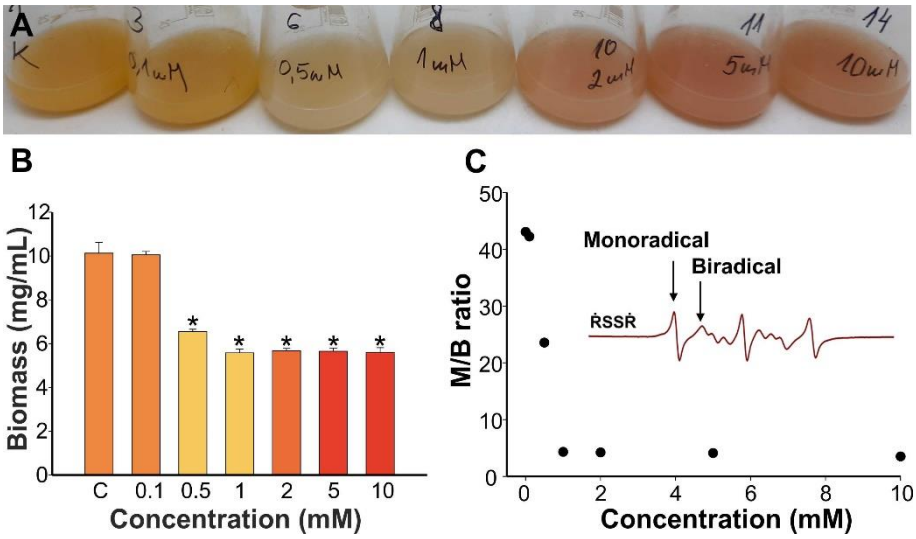
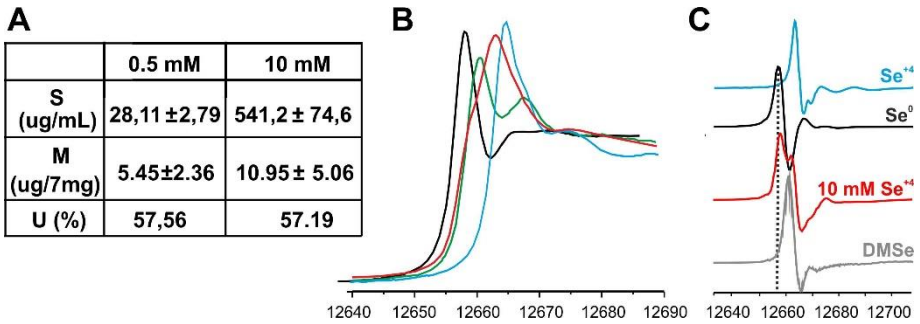


Figure 1. A. 28 h old mycelium treated for 24.h with increasing concentrations of Se^{+4} **B.** Biomass of mycelium after 28 h treatment with increasing concentrations of Se^{+4} **C.** EPR determination of thiol content *in vivo* – y axes shows ratio of mono- and biradical peak of RSSR probe

For further experiments, 0.5- and 10 mM Se^{+4} treatments were selected as low (no visible SeNP production) and high (visible SeNP production) concentrations. ICP-OES measurements (**Fig 2A**) show deficiency in measured Se in relation to the amount added, and it is presumed that at least some of this amount can be contributed to volatile methyl-selenides. The spectrum of mycelium incubated 24 h in 0.5 mM Se^{+4} reveals reduction of Se^{+4}, indicated by red shift of absorption edge compared to

Se^{+4} standard. (**Fig 2B**). The post-edge region contains additional peak at 12667 eV , typical for XANES of RSSeSR containing organic molecules [6, 7]. Yu et al. (2018) have found that sulfhydryl sites of bacterial cell envelope promote Se^{+4} reduction to neutral RSSeSR compounds that can be more easily transported across cell membrane. For incubation with 10 mM Se^{+4} for 24h, the shoulder at 12658.5 eV appeared as a new component in spectrum (**Fig 2B**), indicating reduced form of Se different from those produced in samples treated with lower Se^{+4} concentration [7], most probably originating from red elemental Se, whose presence was already visually confirmed. The main peak of the spectrum positioned at 12663 eV can originate from methylated Se^{-2} compounds. Comparison with DMSe standard gave a good match (**Fig 2C**).



	0.5 mM	10 mM
S (ug/mL)	28,11 ±2,79	541,2 ± 74,6
M (ug/7mg)	5.45±2.36	10.95 ± 5.06
U (%)	57,56	57.19

Figure 2. A. ICP-OES quantification of Se in supernatant (S) and mycelium (M) of fungi treated with 0,5 and 10 mM Se^{+4}. U – selenium quantity that is unaccounted for. Se content in biomass was expressed as µg/7mg, as in this stage of the growth curve, there is 7 mg_{DW} per 1 mL of supernatant **B.** XANES spectra of mycelium treated with Se^{+4}; reference standards of Se^{0} (black), Se^{+4} (blue) vs. mycelium incubated with 0.5 mM Se^{+4} (green) and 10 mM Se^{+4} (red) for 24 h. **C.** the first derivative spectra of Se^{0}, Se^{+4} and DMSe reference standards vs mycelium treated for 24 h with 10 mM Se^{+4}

CONCLUSION

Mycelium of *P. blakesleeanus* reduces selenite to at least two forms, Se^{0}, in the form of SeNPs, and Se^{-2}, in the form of volatile DMSe [8]. Availability of thiols from proteins or glutathione decrease during this process, which could be a consequence of their direct involvement in Se^{+4} reduction, or through neutralization of ROS, but most probably both.

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